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LASER DOPPLER MEASUREMENT OF CUTANEOUS BLOOD FLOW

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This work describes an instrument for the noninvasive measurement of cutaneous blood flow velocity. The system utilizes the Doppler shift of laser light backscattered from moving red blood cells in the cutaneous microcirculation, the shift being obtained by an optical heterodyning technique. Comparison is made between this technique and the ^{133}xenon clearance technique in measuring cutaneous flow in the forearms of normal volunteers. Variations in flow were obtained by inducing different degrees of solar erythema with an ultraviolet sunlamp. A Y on X linear regression yielded a regression coefficient = 0.89 ($p < 0.001$, $n = 16$) between the two methods. The laser Doppler method appears to represent a practical technique for clinical evaluation of cutaneous blood flow in any skin surface.

Noninvasive measurement of blood flow through vessels both large and small has long been both a clinical and research goal. A variety of techniques and instruments have been devised depending upon the degree of exposure of the tissue in which flow is being measured. These range from the application of plethysmography and calorimetry to the intact skin to electromagnetic and ultrasonic systems for evaluation of flow in exposed vessels. Although each of these techniques have advantages in given situations, there are drawbacks to all of them. Not the least of these is that they tend to measure either large vessel or total flow, and are unable to define regional or microcirculatory flow. This type of flow can be measured using radioactive tracers, but these methods are invasive, although only minimally, and do require radioactive exposure. The object of this report is to describe a technique using coherent laser light with optical mixing to measure flow velocity through the superficial microvascular system, and to compare it with the ^{133}xenon clearance technique of measuring cutaneous blood flow.

The technique is based on the measurement of the Doppler frequency shift in monochromatic laser light which is backscattered from moving particles, in this case red blood cells. Because the red cells are moving at different velocities, the single laser output frequency is shifted to a spectrum of different frequencies, and is usually measured in terms of the broadening of spectrum. Yeh and Cummins [1] described the use of this technique to measure the flow velocity and determine the velocity profile of a dilute colloidal suspension in a large-diameter tube, and were able to resolve velocities down to 0.007 cm/sec. This method used optical heterodyning which involved mixing a reference beam with light scattered from the moving target on the surface of an optical detector where they "beat" together and produced a frequency proportional to the Doppler-shifted frequency. Riva, Ross, and Benedek [2] applied this to biologic systems, measuring blood flow through 200-μm-diameter capillary tubes and demonstrated a linear relationship between the maximum shifted frequency and the observed flow. They then applied this to flow velocity measurement in the retinal artery of a rabbit, and subsequently Tanaka, Riva, and Ben-Sira [4] used a fiberoptic catheter in the system to determine the flow velocity intraluminally in the vein of a rabbit.

Stern [5] made initial observations on the feasibility of using this technique to measure the cutaneous microcirculation, and demonstrated, with us, close correlation between the laser Doppler and the ^{133}xenon clearance method [6] of estimating skin blood flow (unpublished observations). This present system utilizes the above principles, but is redesigned to be a practical clinical instrument.

MATERIALS AND METHODS

Laser Doppler System

A block diagram of the configuration is illustrated in Figure 1. A 5-mw, continuous wave, helium–neon laser (Spectra-Physics model 120) is used as the light source. The output is focused by a lens on a 6-mil (0.006-inch) fiberoptic fiber, which carries the light to the skin surface. This light is reflected from both the nonmoving skin surface and the moving red blood cells. The reflected light, consisting of the nonshifted "reference" beam, and the Doppler shifted signal, is gathered by a 30-mil (0.030-inch) fiberoptic fiber and transmitted to the face of a photodiode (EG + G model SGD100A). These two signals are mixed (optically heterodyned) on the face of the photodiode, and "beat" together at a frequency proportional to the Dop-

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Abbreviations:
RMS: root mean square
UV: ultraviolet

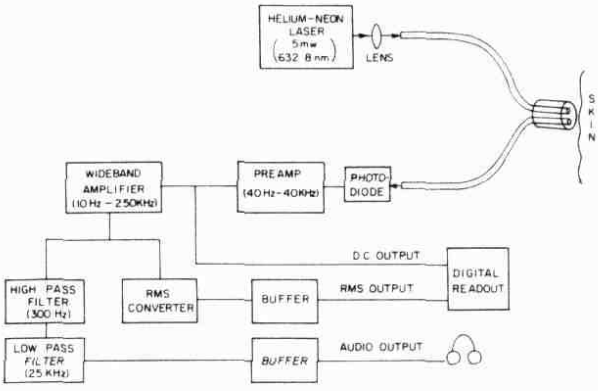

FIG. 1. Block diagram of laser Doppler system.

pler shift, a frequency shift of 30 kHz being equal to a velocity of 0.5 cm/sec. The output current of the photodiode is proportional to the beat-frequency spectrum.

To obtain a single output value for the spectrum, the root mean square (RMS) value is used. The output current from the photodiode containing the spectral information is fed into an RMS converter (Burr Brown #4341). The output signal from the converter is composed of a true velocity component plus a noise component generated by the RMS converter, this latter varying with the intensity of the backscattered, unshifted light. An unshifted light input intensity is recorded at the time of each flow determination. To determine the RMS value for this noise, a plot of the unshifted light (DC) input to the RMS converter vs the output of the RMS converter is made. Thus, for a flow measurement, the RMS noise value for each DC input value is read from the graph and subtracted from the total RMS value to give RMS flow. These values are internally consistent but are arbitrary with respect to flow velocity.

Experimental

Blood flow in the forearm of normal subjects was measured both by the ^{133}xenon clearance and laser Doppler techniques. The subjects, 2 men and 3 women, were normal volunteers between the ages of 21 and 53 years. The subjects were placed at supine bed rest for 20 min in a room with the temperature controlled at 22 ± 0.2°C. Skin temperature was measured by thermistors attached to the volar forearm skin and did not vary more than 0.2°C throughout the measurements. A vacuum splint enclosed the lower half of the circumference of the arm from the wrist to the elbow for stability.

Xenon disappearance was measured by the injection of 30 μc ^{133}xenon dissolved in 0.02 ml of sterile, pyrogen-free physiologic saline into the skin using a 30-gauge needle and Hamilton microsyringe [6, 7]. The rate of ^{133}xenon clearance from the skin was detected by a gamma detector using a 2-inch NaI crystal-photomultiplier detector. The output was recorded for 200 sec both on punched paper tape for computer analysis and as a semilogarithmic plot on a ratemeter-strip chart recorder. Flow was determined as previously described [6] according to the Kety treatment of the Fick flow equation with results expressed in ml/min/100 gm of tissue.

Laser Doppler flow velocity was measured by placing the fiberoptic probe just touching the skin surface using a micromanipulator. RMS and DC values, when stable, were read from the digital display, the backscattered intensity and noise correction computed and subtracted, and the corrected RMS value averaged for each area.

Two areas, approximately 5 × 5 cm, were identified on the volar forearm of both left and right arms. The most proximal area on each arm was irradiated with ultraviolet light (UV) with a sunlamp (Westinghouse model #RSK/1) at 12 inches for 8 to 10 min between 16 and 24 hr prior to the time measurements were made in order to induce erythema and an increased flow. At the time of flow measurement, a circle 2.4 cm in diameter was made in the center of both the UV-irradiated and nonirradiated areas on both arms. Five smaller circles, 2 mm in diameter, were drawn within the larger circle in the pattern of the dots on the "five face" of a die. Measurements were first made with the laser Doppler in each of the five areas. The xenon was then injected into the centermost of the five areas, and the clearance flow measured. The laser Doppler flows were then again recorded in each of the five areas, and the values before and after xenon flow determinations averaged.

RESULTS

Two hundred laser Doppler and 20 ^{133}xenon clearance measurements were made in 5 subjects. The laser Doppler instrument proved easy to use and permitted rapid reproducible measurements in any cutaneous area. Observations showed increased flow in erythematous areas, and a flow velocity approaching the zero flow of a nonmoving surface when a blood pressure cuff occluded the flow to the extremity. Local variations in flow were noted as the sensor was moved across the skin surface.

A plot of the laser Doppler flow velocity vs the ^{133}xenon clearance flow is illustrated in Figure 2. Confluent, diffuse erythema was seen in the UV-irradiated areas in all cases, giving a wide range of flow values. Xenon injections were made at excessive depth in two determinations in one subject giving artifically low flows. Xenon flow data from this subject were therefore excluded, but the laser Doppler data retained. These data were analyzed using a Y on X regression with a least squares best fit straight line giving the regression coefficient, $r = 0.89$ ($p < 0.001$). The laser Doppler flow velocity values represent the mean of the "before" and "after" xenon-measured values. The value measured at the xenon injection site in nonirradiated areas after the injection was not included in this, however, as it demonstrated a consistent elevation with a mean of 81% over the control value. No significant difference was seen

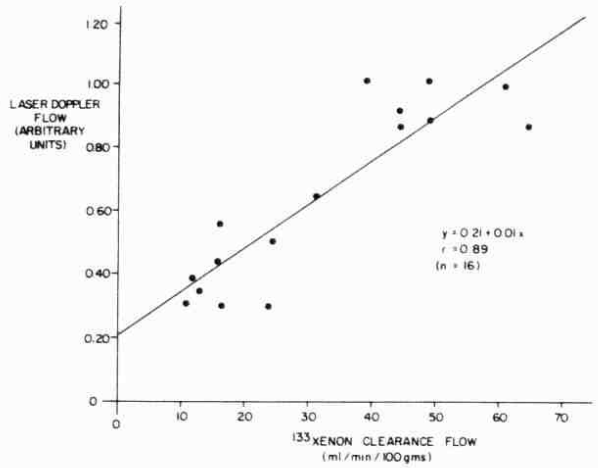

FIG. 2. Laser Doppler flow vs ^{133}xenon clearance flow. The laser Doppler flow is in arbitrary units; the ^{133}xenon clearance flow in ml/min/100gm.

at the injection site in the erythematous, UV-irradiated areas. Maximum values of hyperemic flow in the erythematous areas represented an increase in flow of 134% as compared to mean flows in nonerythematous areas.

DISCUSSION

The utility of the laser Doppler system for the measurement of cutaneous blood flow rests both in its accurate representation of flow through the skin, and its ease of use in a clinical situation, for which the current system was designed. In these studies, it proved simple and quick to set up, and a series of measurements could be made rapidly and easily. Only a minimum of experience was required to be effective in its utilization.

Determination of the accuracy of the flow measurement is a more complex problem. The various methods of cutaneous blood flow measurement currently in use measure a number of different parameters which are felt to be more or less directly proportional to flow, for example volume changes in plethysmography or heat transfer in calorimetric methods. None is without its shortcomings. We had chosen to use the ^{133}xenon clearance technique because of its demonstrated good correlation with another method, venous occlusion plethysmography [6], and because of our extensive experience with the technique [7–9]. In interpretation of the results, however, it must be recognized that each actually measures a different parameter. Radioisotope tracer is taken up by the microcirculation, but depends on injection depth and diffusion of the tracer through the tissue between the injection site and the capillary. ^{133}Xenon is probably not diffusion limited at these flows, but is very fat soluble which can make the clearance data analysis more complex. The laser Doppler technique measures the velocity of moving objects, in this case basically the red cells, and therefore does not give a direct measure of flow. Furthermore, the effective depth of laser penetration is approximately 1 to 1.5 mm, whereas the xenon will be cleared at whatever depth it is injected.

However, as flow is equal to velocity times the cross-sectional area through which the particle is moving, and if the cross-sectional area of flow remains constant, the velocity measured will be proportional to the flow. The area which is illuminated by the laser is large compared to the cross-sectional area of individual capillaries, and the total capillary cross-sectional area within it may not vary too greatly. If this is the case the mean velocity would maintain a proportional relationship to flow. We have shown here that a close relationship does exist between the velocity and flow measurement techniques at varying flows and supports the concept that the mean cross-sectional area may be relatively constant.

The close correlation between the two methods additionally supports the value of the laser Doppler as a clinically useful instrument. This value lies in the fact that it is noninvasive, and that flow velocity can be recorded under changing conditions in any skin area of the body. It could thus prove its value in a number of clinical and research situations, for example, in evaluating the viability of skin grafts, evaluating the level of peripheral cutaneous flow in patients with arteriosclerosis, and assessing the effects of both topically and systemically administered medications.

Additionally of note is the increase in flow measured by the laser Doppler which occurred at the injection site following ^{133}xenon injection. Although hyperemia due to this "trauma of injection" had been previously suspected, no technique was available that permitted its actual measurement. In this case, 10 determinations in nonerythematous, control areas reflected a mean increase of 81% in flow velocity approximately 5 to 7 min following the xenon injection. In comparison, in the UV-irradiated erythematous areas where flow was already increased, no significant increase in flow was noted, as was also true in control, noninjected areas. Although this can represent an error of some magnitude, the radiotracer clearance technique should not be categorically dismissed. It still remains one of the only techniques for measuring flow in any area of the body, and it is unknown what magnitude of error is associated with various other techniques.

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